noses of symptoms, signs, and ill-defined conditions. Joint pain, osteoarthrosis, and backache were the most common diagnoses among those of musculoskeletal and connective tissue diseases.

According to the report, "to date, there is not clinical evidence for a previously unknown, serious illness or 'syndrome' among Persian Gulf veterans participating in the study. A unique illness or syndrome . . . would probably be detectable in the population of 18,598 patients." These results concur with the conclusions of a National Institutes of Health Technical Assessment Workshop on the Persian Gulf Experience and Health held in April 1994, where a panel of nonfederal experts found that "no single disease or syndrome is apparent, but rather multiple illnesses with overlapping symptoms and causes." The report also points out that the study was limited and cannot be sufficiently generalized to other veterans due to factors such as self-selection of patients, recall bias, inability to validate self-reported exposures, and the lack of an appropriate control group.

A recent study, entitled *Neurotoxicity Resulting from Coexposure to Pyridostigmine Bromide, DEET, and Permethrin: Implications of Gulf War Chemical Exposures*, has linked the combination of these chemicals to neurological damage. The study, led by Mohamed Abou-Donia, a professor of pharmacology at Duke University, was published in the May issue of the *Journal of Toxicology and Environmental Health*, and may have implications for research on a Gulf War syndrome.

Abou-Donia's team researched the neurological effects of the antinerve agent pyridostigmine bromide, the insect repellent DEET, and the insecticide permethrin on hens, which are recommended by the EPA for neurological testing because their nervous system resembles that of humans. The study found that, when administered individually, the compounds result in minimal toxicity, but when combined, they produce adverse neurological effects.

The hens were given doses of the chemicals that were three times the doses received by Persian Gulf soldiers. A group of hens given the chemicals individually suffered from minor effects. However, other groups of hens given two of the chemicals together developed diarrhea, weakness, shortness of breath, and inability to fly correctly. According to Abou-Donia, some of these symptoms were similar to those experienced by the veterans. Some of the hens in groups that were exposed simultaneously to all three chemicals became paralyzed or died.

Researchers hypothesize that the combination of chemicals results in a failure of the body's ability to neutralize them. "The mixture of chemicals seems to decrease the ability of the body to rid itself of the chemicals and detoxify itself," said Abou-Donia. The researchers suggested that the antinerve-gas drug may have inhibited the production of the enzyme butyrylcholinestarase (BuCHe), which breaks down nitrogen-containing organic compounds such as the insect repellents. When the enzyme was blocked, the other chemicals were allowed to enter the brain, thus causing neurological damage.

Abou-Donia is currently looking closer at how the mixture of chemicals may have caused neurological damage. He also plans to study blood samples from the veterans for levels of BuCHe, other enzymes, and other markers.

This study raises questions about multiple chemical exposures, an area that has not been thoroughly studied. Questions also remain about the reproductive effects of the veterans' exposures. Many veterans have reported experiencing reproductive problems since the war. According to the DOD study, however, "these reports have not been validated [by a] review of medical records or other sources of information." However, the report says, this is an important issue that the DOD will study further.

Deep Sea Microbes and DNA Cloning

Scientists are using microbes from ancient environments to enhance modern biotechnology. DNA polymerases from archaea, microorganisms that thrive in hydrothermal vents, make longer, more accurate DNA copies and work at higher temperatures in the polymerase chain reaction (PCR) than a commonly used bacterial enzyme.

Some archaea survive at above 100°C in the high-pressure, deep-sea vents that represent Earth's earliest environments, says John Baross, professor of oceanography at the University of Washington in Seattle. Baross collects archaea from the Pacific Ocean at depths of 2,000 meters or more, where seafloor spreading causes magma-heated fluids to spew forth. He has cultured about 300 isolates of *Thermococcus* and other hyperthermophilic archaea, which grow best at temperatures of 85–113°C.

Baross is seeking new enzymes to improve the efficiency of research and industrial reactions. PCR, for example, employs cycles of heat and cooling to denature and polymerize DNA. "What we're looking for in a new DNA polymerase is something that can amplify templates to greater than 10,000 bases, that has a very high fidelity with a variety of primers, and is more thermally stable," Baross says. Enzymes from high-temperature archaea have the advantage of withstanding cycling longer than the commonly used *Taq* polymerase, which is derived from the bacterium *Thermus aquaticus*.

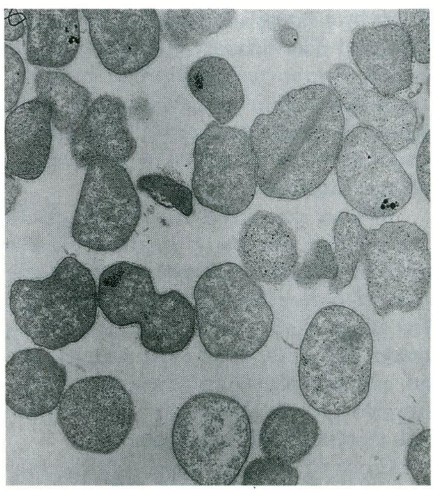
Archaeal polymerases also have proofreading, or exonuclease, activity. They chew off mismatched bases as DNA is polymerized, in some cases making copies 10-fold more faithful to the original than *Taq*. But, cautions Wayne Barnes, an associate professor of biochemistry and molecular biophysics at the Washington University School of Medicine in St. Louis, the same function can retard PCR by degrading DNA primers.

Barnes found that mixing *Taq* and archaeal enzymes balanced proofreading and polymerization. The blend, now being marketed for "long PCR," makes DNA strands of 35,000 bases or more, rather than the 3,000 or so bases typically yielded by *Taq* alone, Barnes says. Archaeal polymerases with various features are now being sold by companies including New England Biolabs, Stratagene Cloning Systems, and Boehringer Mannheim.

Other archaeal enzymes are being tested for processing food and improving flow during oil drilling, says Robert Kelly, a professor of chemical engineering at North Carolina State University. But there may be drawbacks to using them because industrial applications can require tons of enzymes, which archaea don't produce readily in culture. The microbes are anaerobic, often grow slowly, and have unusual nutrient requirements; in addition, their enzymes are difficult to purify, Kelly says.

While archaea resemble bacteria, they're more closely related to eukaryotes, Baross writes in a new book, *Advances in Protein Chemistry* (in press). "With the link between high-temperature archaea and higher organisms," he says, "we may be able to ask questions like 'What is the origin of viruses?,' or 'What is the origin of certain genes?' "

Archaea may also offer clues to finding



James F. Holden

Promising primitives. A strain of deep sea microbes called *Thermococcus* are improving DNA cloning.

other life in the solar system. "The first microbial ecosystems on Earth probably occurred in the sub-seafloor associated with hydrothermal venting," Baross says. "A number of planets underwent parallel evolution [physically and hydrologically] . . . so similar life may have arisen on Mars, Venus, or a number of moons."

Free Radicals and Breast Cancer

The metastasis of breast cancer may be the result, at least in part, of damage to tumor-suppressing genes or activation of oncogenes by the hydroxyl free radical, according to results of a study by scientists at the Pacific Northwest Research Foundation in Seattle.

A free radical is a molecule with an unpaired electron. The hydroxyl radical (-OH) results from the metabolism of hydrogen peroxide (H_2O_2), probably a by-product of the cycling of estrogen. Normally, the free radical is countered by the cells' antioxidant systems without damage to the cellular DNA. As a woman ages, however, cellular protection may become less effective and DNA damage is wrought faster than the free radicals can be controlled and damage repaired. This process also can be hastened by exposure to radiation, carcinogenic chemicals, and high-fat, high-calorie diets.

Building upon their previous finding of significantly higher amounts of DNA damage in women with breast cancer than in healthy women, Donald C. Malins, director of the Molecular Epidemiology Program at the foundation, and colleagues began to assess what they termed radical-induced DNA disorder (RIDD).

Malins obtained 12 cancer tissue specimens from women with breast cancer who had no nodal evidence of metastasis; 25 specimens from women with metastatic cancer; and 21 specimens of breast tissue from healthy women who had undergone breast reduction surgery. DNA was isolated from each specimen and analyzed by Fourier transform infrared spectroscopy and gas chromatography to determine the extent of DNA damage. Results, reported in the March 19 issue of the *Proceedings of the National Academy of Sciences*, showed that the DNA from the metastatic tissue had more than twice the damage of the DNA from the nonmetastatic tissue, and also had a greater diversity in the base DNA structure.

"It appears that free radicals play a major role in causing genetic changes linked to cancer and metastasis," Malins says. "RIDD may form the basis of a test to determine a woman's risk of metastatic cancer and perhaps enable some intervention to prevent it."

Once the cancer has formed, Malins pointed out, it begins to produce its own H_2O_2, leading to reduced cell adhesion within the tumor, allowing cells to be shed and to migrate and colonize elsewhere, and producing higher levels of -OH. "Getting an antioxidant into the tissue to reduce the hydroxyl level could be instrumental in controlling the cancer," he said.

Some scientists have questioned the methodologies used by Malins and note that studies published in the February 1996 issue of *Carcinogenesis* and the December 1995 issue of *Chemical Research in Toxicology* failed to duplicate Malins' initial results using identical methods.

However, "this is a logical step forward in determining the role of free radicals in carcinogenesis and metastasis," said John A. Strupp, director of oncology and hematology at St. Thomas Hospital and clinical professor of oncology at Vanderbilt University School of Medicine in Nashville. "Whether intervention with antioxidants such as vitamins A, E, and C can reduce free-radical production and cancer metastasis remains to be seen."

EHPnet

Seafood and Health

Satisfying the world's appetite for seafood requires that 89 tons of fish be harvested every year. Though many think of seafood as a healthy alternative to meat, aquatic pollutants and epidemics in some fish-producing countries are threatening the quality and safety of much of the world's seafood supply. In an attempt to ensure that the seafood Americans eat is free from toxic chemicals and pathogens, the FDA has developed new regulations, including the Hazard Analysis and Critical Control Point (HACCP) program, to govern seafood production and distribution.

SeafoodNIC

New health threats and regulations can become confusing for both consumers and people in the fishing industry. To help simplify matters, Robert J. Price of the University of California–Davis has developed the Seafood Network Information Center, or SeafoodNIC, on the Internet. The site, located at http://www-seafood.ucdavis.edu, contains a coherent abundance of information concerning fish, potential health risks associated with consuming fish, and relevant regulations. From the home page, users can access information on upcoming seafood symposia and meetings around the world, detailed information about HACCP (though this section is largely unfinished), texts of various FDA regulations, a collection of papers on seafood safety and quality, and a long list of links to other seafood-related sites.

The Guidelines and Regulations link connects users to the bulk of the health-related information available at the site. This section contains descriptions of new rules proposed by the FDA as well as the text of current regulations. Some of the posted regulations are also provided in a format that can be downloaded to a word processing program. Under the Model Forms, Guides, and Lists heading of the 1993 FDA Food Code, a page is maintained that lists fish-producing regions of the world with data on what diseases are epidemic or endemic in those areas according to the World Health Organization and the Centers for Disease Control and Prevention. The list reflects the growing concern that human pathogens like *Salmonella typhi* and the hepatitis A virus can be transmitted worldwide through exported food products. The 1994 FDA Hazards and Control Guides section includes a list of popular commercial fish species along with the health hazards commonly associated with each of them. A similar list is provided for health hazards associated with particular steps in seafood processing. Both lists take into account chemical pollutants present in fish as well as microbial contamination. In a separate section, an explanation of each contaminant is provided along with information on how to protect fish products from it; pathogens, mercury, natural toxins, decomposition, histamines, food additives, aquaculture drugs, parasites, and chemical contamination hazards are all covered in this section.

Under the heading Links and Information Services, users can access additional information from related sites. The extensive list of linked sites includes fishing industry associations, agencies of the federal government, marine sciences libraries, professional societies, seafood industries, Sea Grant programs, and university food science programs.

To keep even closer tabs on developments in the areas of fish and human health, users can join the SeafoodNIC newsgroup via e-mail by following the instructions provided under the heading Seafood Listserv.